High Level of Plasma Remnant-like Particle Cholesterol May Predispose to Development of Hypertension in Normotensive Subjects

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ABSTRACT

BACKGROUND

Remnant-like lipoprotein particle cholesterol (RLP-c) is a highly atherogenic factor. RLP-c induces endothelial dysfunction and is associated with hyperinsulinemia. The present study was designed to determine whether high plasma RLP-c levels predispose to the development of hypertension in subjects with normal blood pressure (BP).

METHODS

A total of 1,485 subjects aged over 40 years in a Japanese Cohort of the Seven Countries Study received health examinations. We examined BP, anthropometric parameters, and blood chemistries including fasting RLP-c levels. RLP-c levels were measured by an immune-separation method. We excluded from the analysis 676 subjects who had hypertension (BP≥140/90mmHg), or were on antihypertensive medication, and/or on antihyperlipidemic medication at baseline. Ten years later, 681 subjects were re-examined.

RESULTS

Of 681 normotensives at baseline, 303 subjects had developed hypertension 10 years later. Baseline RLP-c level was significantly higher ($p<0.01$) in

the subjects who developed hypertension than in those who remained normotensive (3.7±1.9 vs. 3.3±1.6 mg/dl). Multivariate logistic regression analysis demonstrated that baseline RLP-c was a significant factor for incident hypertension after adjustments for homeostasis model assessment (HOMA) index and other hypertension related factors [odds ratio, 1.05 (95% CI; 1.00-1.10); p=0.04].

CONCLUSION

A high level of plasma RLP-c in normotensive subjects may predispose to the development of hypertension in a population of community-dwelling Japanese.

Keywords**:** *lipoprotein; blood pressure; prospective study; epidemiology*

INTRODUCTION

Remnant-like lipoprotein particle cholesterol (RLP-c) derived from triglycerides-rich lipoprotein is taken up by macrophages and causes foam cell formation in vitro,[1] and is considered to be highly atherogenic.[2-4] Elevated plasma RLP-c levels are associated with endothelial dysfunction[5-9] and hyperinsulinemia.[10,11] Endothelial dysfunction and hyperinsulinemia may be associated with the development of hypertension.[12-14] Thus, it is considered that elevated plasma RLP-c levels may be associated with incident hypertension. In fact, elevated RLP-c was associated with hypertension in a general population without diabetes[15] and in patients with preeclampsia.[16] However, because of the cross sectional nature of the previous studies, it was not clear whether high RLP-c levels in subjects with normal blood pressure (BP) is associated with incident hypertension. Accordingly, we conducted a longitudinal study in subjects in a general population without hypertension at baseline to evaluate the relationship between circulating RLP-c levels and incident hypertension.

METHODS

Study population.

A periodic epidemiological survey was performed in 1999 in a rural farming community located in the south-west part of Japan (Tanushimaru town). An earlier cohort in Tanushimaru was a part of the Seven Countries Study.[17] As reported previously, the demographic backgrounds of the subjects in this area were similar to those of the general Japanese population.[18] We examined 1,485 persons (602 males and 883 females) over the age of 40 years. We excluded 678 subjects from the analysis who had hypertension (BP≥140/90mmHg) and/or were on antihypertensive medications or on antihyperlipidemic medications, and/or had RLP-c ≥100mg/dl (n=2) at baseline.

A cross-sectional analysis was performed in 807 subjects. Ten years later, in 2009, we conducted a follow-up examination in the same cohort. Of 807 subjects, 65 (8.1%) had died, and 61 (7.6%) refused the re-examination. Consequently, 681 subjects (248 males and 433 females) were re-examined 10 years later and enrolled in this study.

Data collection

A general medical history including smoking, alcohol intake and current medication, was obtained. Height and weight were measured, and body mass index (BMI) was calculated as an index of obesity. Waist circumference was measured at the level of the umbilicus in the standing position. BP was measured twice with the subjects in the supine position. The second BP was adopted and systolic and fifth-phase diastolic pressures were used for analysis. Blood was drawn from the antecubital vein in the morning after a 12-h fast for determinations of lipids profiles (RLP-c, triglycerides, high-density lipoprotein cholesterol [HDL-c], and low-density lipoprotein cholesterol [LDL-c]), fasting plasma glucose (FPG), insulin, hemoglobin A1c [HbA1c (NGSP)], blood urea nitrogen (BUN), creatinine, von Willebrand Factor (vWF), and fibrinogen levels. Fasting blood samples were centrifuged within 1 hour after collection. Creatinine was measured by enzymatic method. vWF was measured by a double-antibody ELISA and fibrinogen was measured by high-performance liquid chromatography (HPLC). Serum RLP-c was measured by immune-separation technique[19] (using an immune-affinity gel containing monoclonal antibodies to human apolipoprotein [apo] B-100 and apo A-1).

Five milligrams of anti-apo B-100 and anti-apo A-1 IgG each were coupled to 1 ml of CNBr-Sepharose 4B gel, according to the recommendations of the manufacturer. Then, the remaining active sites on the gels were blocked with 0.2 M glycine. Before use the gels were conditioned with calf serum for 1 hour at room temperature with gentle shaking to reduce non-specific binding and washed with 1.0 M acetic acid/0.5 M NaCl. This procedure was repeated twice.[19] Intra- and inter-assay coefficients of variation of RLP-c in the commercially available laboratory that performed the assays (The Kyodo Igaku Laboratory, Fukuoka, Japan) were 7.6% and 7.8%, respectively. A homeostasis model assessment (HOMA) index [FPG (mg/dl) × insulin (μU/ml)/405] was calculated from fasting glucose and insulin levels as a marker of insulin resistance.[20] Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation modified with a Japanese coefficient[21]: eGFR (mL/minute/1.73m^2) = 194×Age$^{-0.287}$×Serum creatinine$^{-1.094}$ (if female×0.739). Hypertensives were defined as those with BP≥140/90mmHg and/or those receiving antihypertensive medication.

This study was approved by the Tanushimaru branch of the Japan

Medical Association and by the mayor, as well as by the ethics committee of Kurume University School of Medicine. All participants gave informed consent.

Statistical analysis.

Results are presented as means ± standard deviation (SD), unless otherwise specified. Because of skewed distributions, natural logarithmic transformations were performed for RLP-c, triglycerides, insulin and HOMA index. These variables were represented as original scale after analysis by log (natural) transformed values in tables. In Tables 1, 2 and 3, values of RLP-c, triglycerides, insulin and HOMA index are presented as geometric mean and range. The χ^2 test was used for categorical parameters to test differences between groups. Sex, smoking habits, and alcohol intake were used as dummy variables. Univariate logistic regression analysis was performed for factors related to the development of hypertension. We estimated odds ratios and their 95% confidence intervals per 1-unit (approximately 1 SD) increase in the variables. The odds ratios for RLP-c, triglycerides, insulin and HOMA index are the odds ratio per exp (SD) time

increase of the levels. Multiple logistic regression analysis was performed to determine independent parameters for development of hypertension after adjusting for age, sex, and confounding factors. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using the SAS system (Release 9.3; SAS Institute, Cary, NC).

RESULTS

Table 1 shows the baseline characteristics of the 681 normotensive subjects in 1999 stratified by gender. As is apparent from the table, the enrolled subjects had almost normal mean values of risk factors and other variables. Mean RLP-c level at baseline was 3.4 ± 1.8mg/dl. RLP-c level was significantly higher ($p<0.01$) in males than in females. **Table 2** shows results of uni- and multi-variate regression analyses for the cross-sectional correlates with baseline RLP-c levels. Multivariate analysis showed that HOMA index, LDL-c, HDL-c (inversely), total cholesterol/HDL-c ratio and triglycerides were significantly associated with RLP-c. **Table 3** shows univariate logistic regression analysis performed for predictors of the development of hypertension in 10 years. Age, male gender, RLP-c,

systolic and diastolic BPs, BMI, waist circumference, HOMA index, and triglycerides were significantly associated with the development of hypertension. **Table 4** shows multivariate logistic regression analysis performed for predictors of the development of hypertension after adjustments for confounding factors. A significant (p=0.04) odds ratio for plasma RLP-c level was observed in the final model (model 5) [1.05 (95% CI: 1.00-1.10)] for the development of hypertension (adjusted for baseline values of age, sex, systolic BP, BMI, waist circumference, HOMA index and triglycerides).

DISCUSSION

Based on known aspects of the function of plasma RLP-c, we hypothesized that a high level of RLP-c could predict the development of hypertension. RLP was an independent predictor for the development of hypertension after adjustments for other lipid profiles. Our results are consistent with our hypothesis that high levels of RLP-c were associated with the development of hypertension after 10 years in normotensive subjects at baseline. This study is the first to demonstrate a significant association between high plasma levels of RLP-c and the development of hypertension in a general population.

Several mechanisms could explain the association between RLP-c and development of hypertension. Because hypertension is associated with endothelial dysfunction and because preexisting endothelial function will precede hypertension, RLP-c induced endothelial dysfunction may be one such mechanism.[5-9] In order to examine the contribution of endothelial dysfunction to the development of hypertension, we measured two serum markers of endothelial dysfunction, von Willebrand factor[22,23] and fibrinogen.[24,25] Our results demonstrated no contribution of these two

factors (**Table 3**). However, from the present study, we cannot rule out the possibility that endothelial dysfunction induced by RLP-c contributed to the development of hypertension because we had no data from more direct and reliable measures for endothelial dysfunction like responses to acetylcholine measured by the venous occlusion plethysmograph.[26-29]

Prospective studies[13,14] have shown that increased insulin concentration predisposes to the development of hypertension. Hyperinsulinemia causes sympathetic activation by acting on the brain and impairs sodium secretion from the kidney,[30] leading to hypertension.[31] It has been reported that elevated RLP-c levels are associated with hyperinsulinemia or insulin resistance.[10,11,32,33] In fact, our multivariate analysis of the baseline data demonstrated a significant association between RLP-c and HOMA index, a marker of insulin resistance. (**Table 2**). Thus, another possible explanation of the relationship between RLP-c and development of hypertension may be hyperinsulinemia or insulin resistance. In this study, we determined baseline factors associated with the development of hypertension. Besides well-known factors like age, BP and obesity, hyperinsulinemia and/or insulin resistance were significant factors

for the development of hypertension (**Table 3**), consistent with previous reports.[13,14,34] Thus, our study appears to suggest that insulin resistance may underlie the association between RLP-c and the development of hypertension. However, our detailed analysis denied this possibility and suggested that RLP-c predicted the development of hypertension, independent of insulin resistance (**Table 4**).

The issue of lipid control and risk of hypertension is interesting. Meta-analysis of 20 randomized, controlled trials showed a small but statistically significant reduction of systolic BP with statin therapy.[35] Some review articles also supported the notion that statin may reduce blood pressure.[36,37] Moreover, it was reported that bezafibrate reduced blood pressure in patients with hypertriglyceridemia.[38] Although it was not reported whether the blood pressure reduction was ascribed to RLP-c reduction, it may be feasible to consider that lipid lowering therapy reduces blood pressure.

Study Limitations and Perspective

The limitations of our study are, first, 15.6% of the subjects were lost to

follow-up because of death or refusal to participate in re-examination. Accordingly, we limited the analysis to subjects for whom all baseline and follow-up data were available. Second, the results of this study do not necessarily apply to other populations of different ethnicity. Third, we performed follow-up examination only once after 10 years. Thus we were not able to do Cox`s regression analysis. A final limitation was the lack of data of plasma RLP-c at follow-up. Nonetheless, this study suggests that plasma RLP-c plays a key role in the development of hypertension. This is the perspective of the present study.

In conclusion, the present study demonstrated that a high level of plasma RLP-c predicted the development of hypertension 10 years later in normotensive subjects at baseline. High level of plasma RLP-c may predispose to the development of hypertension.

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Disclosure:

The authors declared no conflicts of interest.

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Table 1. Characteristics of 681 subjects with normal blood pressure at baseline

Parameters†	Males	Females	Total
Total No.	248	433	681
Age (years)	60.3±10.1	57.6±9.7	58.6±9.9
Sex (%)	248 (36.4)	433 (63.6)	681 (100)
RLP-cholesterol (mg/dl)‡	3.7	3.3	3.4
Range (min-max)	0.2-57.7	0.5-59.4	0.2-59.4
BMI (kg/m^2)	23.0±2.8	22.7±3.2	22.8±3.1
Waist circumference (cm)	80.9±8.2	72.0±7.9	75.2±9.0
Systolic BP (mmHg)	121.7±10.5	118.0±12.1	119.4±11.7
Diastolic BP (mmHg)	75.5±7.8	71.9±8.4	73.2±8.4
Glucose (mg/dl)	98.0±15.8	93.5±13.1	95.1±14.3
HbA_{1c} (NGSP) %	5.4±0.7	5.5±0.7	5.5±0.7
Insulin (μU/ml)‡	4.0	4.7	4.4
Range (min-max)	1-56	1-52	1-56
HOMA index‡	0.97	1.07	1.03
Range (min-max)	0.20-16.3	0.21-12.8	0.20-16.3
LDL-cholesterol (mg/dl)	115.0±28.4	126.8±31.4	122.6±30.8
HDL-cholesterol (mg/dl)	52.5±12.2	59.6±14.7	57.0±14.3
T-chol/HDL-c (mg/dl)	3.8±1.1	3.6±1.0	3.7±1.0
Triglycerides (mg/dl)‡	104.4	86.7	92.7
Range (min-max)	38-963	28-350	28-963
eGFR ($ml/min/1.73m^2$)*	65.0±10.3	63.1±10.2	63.8±10.3
von Willebrand factor (%)	143.3±40.3	137.9±43.0	139.9±42.1
Fibrinogen (mg/dl)	302.7±52.8	311.7±59.3	308.5±57.1
Smoking (n: yes, %)	53 (21.4)	4 (0.9)	57 (8.4)
Alcohol intake (n: yes, %)	146 (58.9)	92 (21.2)	238 (34.9)

*Date are expressed as mean ± SD or %, unless otherwise indicated. Estimated GFR was calculated by the Modification of Diet in Renal Disease (MDRD) study equation.

†Abbreviations; BMI, body mass index; BP, blood pressure; HOMA index, homeostasis model assessment index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T-chol, Total cholesterol; HDL-c, HDL-cholesterol; eGFR, estimated glomerular filtration rate

‡ Log-transformed values were used in analyses.

Table 2. Univariate and multivariate regression analyses for correlates of plasma RLP-cholesterol level at baseline

	Univariate		Multivariate	
Parameters‡	β	p value	β	p value
Age (years)	-0.002	0.56	-0.003	0.44
Sex	-0.082	0.03	-0.014	0.75
BMI (kg/m^2)	0.231	<0.01	0.003	0.74
Waist circumference (cm)	0.264	<0.01	0.002	0.61
Systolic BP (mmHg)	0.072	0.06	0.002	0.21
Diastolic BP (mmHg)	0.050	0.20	0.003	0.10
HbA1c (NGSP) %	0.198	<0.01	0.001	0.96
HOMA index§	0.309	<0.01	0.055	**0.01**
LDL-cholesterol (mg/dl)	0.288	<0.01	0.003	**0.01**
HDL-cholesterol (mg/dl)	-0.286	<0.01	-0.017	**<0.01**
T-chol/HDL-c	0.329	<0.01	0.291	**<0.01**
Triglycerides (mg/dl)§	0.751	<0.01	0.674	**<0.01**
eGFR (ml/min/1.73m^2)$^+$	-0.068	0.08	-0.001	0.41
von Willebrand factor (%)	0.042	0.27	0.001	0.87
Fibrinogen (mg/dl)	0.023	0.55	0.001	0.13
Smoking (yes)	0.148	<0.01	0.009	0.85
Alcohol intake (yes)	0.038	0.32	0.026	0.55

*Males=0, Females=1 R^2=0.58

† Estimated GFR was calculated by the Modification of Diet in Renal Disease (MDRD) study equation.

‡ Abbreviations; BMI, body mass index; BP, blood pressure; HOMA index, homeostasis model assessment index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T-chol, Total cholesterol; HDL-c, HDL-cholesterol; eGFR, estimated glomerular filtration rate

§ Log-transformed values were used in analyses.

The multivariate analysis was performed only for variables found to have statistically significant associations with baseline RLP-c levels in bivariate analysis.

Table 3. Univariate regression coefficients for development of hypertension by a logistic regression analyses

Variable (increment)	β	SE	Odds ratio (95% CI)	p value
Age (10 years)	0.011	0.015	1.02 (1.01-1.04)	**0.01**
Sex (Male)	-0.343	0.160	0.71 (0.52-0.97)	**0.03**
RLP-cholesterol^{+} (1.3 times)	0.086	0.059	1.02 (1.01-1.21)	**0.01**
Systolic BP (11.7 mmHg)	0.060	0.011	1.06 (1.04-1.09)	**<0.01**
Diastolic BP (8.4 mmHg)	0.071	0.014	1.03 (1.02-1.06)	**<0.01**
Body mass index (3.1 kg/m^2)	0.067	0.025	1.02 (1.01-1.21)	**<0.01**
Waist circumference (9 cm)	0.048	0.020	1.06 (1.02-1.10)	**<0.01**
eGFR (10.3 ml/min/1.73m^2)	-0.028	0.040	0.97 (0.90-1.05)	0.49
HbA1c (NGSP) (0.7 %)	0.001	0.003	1.00 (0.99-1.01)	0.91
HOMA index^{+} (1.5 times)	0.293	0.094	1.34 (1.11-1.61)	**0.01**
LDL-cholesterol (30.8 mg/dl)	0.003	0.053	1.00 (0.91-1.11)	0.95
HDL-cholesterol (14.3 mg/dl)	-0.007	0.008	0.99 (0.98-1.01)	0.33
T-chol/HDL-c (1.0 mg/dl)	0.016	0.076	1.01 (0.97-1.37)	0.18
Triglycerides^{+} (1.1 times)	0.006	0.023	1.05 (1.02-1.09)	**0.01**
von Willebrand factor (42.1 %)	0.001	0.002	1.00 (0.99-1.01)	0.91
Fibrinogen (57.1 mg/dl)	0.001	0.001	1.00 (0.99-1.00)	0.67
Smoking	0.241	0.203	1.27 (0.86-1.89)	0.23
Alcohol intake	0.346	0.195	1.41 (0.97-2.07)	0.08

*Abbreviations: SE, Standard error, HOMA index, homeostasis model assessment index T-chol, Total cholesterol, HDL-c, HDL-cholesterol

†These variables are shown in the original scale after analysis using log (natural)-transformed values. The odds ratios for these variables are the odds ratio per exp (SD) time increase of the levels.

Table 4. Odds ratios of plasma RLP-cholesterol levels for development of hypertension

Model	Odds ratio	p value
Unadjusted	1.08 (1.03-1.14)	**0.01**
Model 1	1.07 (1.02-1.12)	**0.03**
Model 2	1.05 (1.00-1.10)	**0.04**
Model 3	1.05 (1.00-1.10)	**0.04**
Model 4	1.05 (1.00-1.10)	**0.04**
Model 5	1.05 (1.00-1.10)	**0.04**

Odds ratio (95%C.I.)

Model 1: Adjusted for age and sex

Model 2: Adjusted for age, sex and systolic BP

Model 3: Adjusted for age, sex, systolic BP, BMI and waist circumference

Model 4: Adjusted for age, sex, systolic BP, BMI, waist circumference and HOMA index

Model 5: Adjusted for age, sex, systolic BP, BMI, waist circumference, HOMA index and triglycerides